Reproductive performance of Lacaune dairy sheep exposed to artificial long days followed by natural photoperiod without and with additional progestagen treatment during the nonbreeding season

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Abstract: This study compared the reproductive performance of Lacaune dairy ewes exposed to a light program and subsequent male introduction without (n = 36) or with (n = 36) an additional 6-day progestagen treatment during the nonbreeding season. All ewes were exposed to extended day length (16 hours light and 8 hours darkness) for 77 days during winter (December 15 until March 2) followed by increasing natural photoperiod. At the end of the photoperiodic treatment, three blood samples were collected 6 days apart for progesterone (P4) analysis to determine cyclic activity. One half of the ewes were additionally subjected to a 6-day progestagen treatment in combination with PGF2 and eCG at insert withdrawal. Rams fitted with marking harnesses were introduced to females for 45 days and marked ewes recorded. Ewes exposed to the light program only were joined 40 days after the end of photoperiodic treatment, and ewes with additional progestagen treatment were joined 1 day after insert removal (40-44 days after the end of photostimulation). Lambing data were recorded and fertility (percentage of ewes lambing, lambing rate, and litter size) assessed to the first service period and overall. Mean serum P4 concentrations were similarly ($P > 0.05$) low in both groups (0.4-0.7 ng/mL vs. 0.4-0.6 ng/mL). On the basis of elevated P4 levels (>1 ng/mL), evidence of luteal activity was found in 27.8% of the ewes at the end of the light program. Estrus response was equally high (97.2%) and estrus distribution highly synchronized in progestagen-treated ewes (91.7% within 4 days). In ewes exposed to the light program only, estrous activity was recorded within 4 days (six ewes), from Day 8 to Day 17 (17 ewes) and from Day 19 to Day 25 (12 ewes) after joining. The percentage of ewes that lambed to the first service period was higher ($P < 0.05$) in ewes exposed to the light program only than that in the group additionally treated with progestagen/PGF2 /eCG (94.4% vs. 69.4%). Overall, the percentage of lambing ewes was similar in both groups (97.2% and 94.4%), and lambing rates (1.4-1.9) and litter sizes (1.9-2.1) were high and not influenced ($P > 0.05$) by the treatment. In conclusion, this study reports that exposition of Lacaune ewes to artificial long days followed by natural day length and male introduction is highly effective to induce fertile estrous activity during the nonbreeding season and offers a reliable and practical alternative to hormonal manipulation for out-of-season breeding in sheep.

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Reproductive performance of Lacaune dairy sheep exposed to artificial long days followed by natural photoperiod without and with additional progestagen treatment during the nonbreeding season

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Fertility

A B S T R A C T

This study compared the reproductive performance of Lacaune dairy ewes exposed to a light program and subsequent male introduction without (n = 36) or with (n = 36) an additional 6-day progestagen treatment during the nonbreeding season. All ewes were exposed to extended day length (16 hours light and 8 hours darkness) for 77 days during winter (December 15 until March 2) followed by increasing natural photoperiod. At the end of the photoperiodic treatment, three blood samples were collected 6 days apart for progesterone (P4) analysis to determine cyclic activity. One half of the ewes were additionally subjected to a 6-day progestagen treatment in combination with PGF2α and eCG at insert withdrawal. Rams fitted with marking harnesses were introduced to females for 45 days and marked ewes recorded. Ewes exposed to the light program only were joined 40 days after the end of photoperiodic treatment, and ewes with additional progestagen treatment were joined 1 day after insert removal (40–44 days after the end of photostimulation). Lambing data were recorded and fertility (percentage of ewes lambing, lambing rate, and litter size) assessed to the first service period and overall. Mean serum P4 concentrations were similarly ($P > 0.05$) low in both groups (0.4–0.7 ng/mL vs. 0.4–0.6 ng/mL). On the basis of elevated P4 levels (>1 ng/mL), evidence of luteal activity was found in 27.8% of the ewes at the end of the light program. Estrus response was equally high (97.2%) and estrus distribution highly synchronized in progestagen-treated ewes (91.7% within 4 days). In ewes exposed to the light program only, estrous activity was recorded within 4 days (six ewes), from Day 8 to Day 17 (17 ewes) and from Day 19 to Day 25 (12 ewes) after joining. The percentage of ewes that lambed to the first service period was higher ($P < 0.05$) in ewes exposed to the light program only than that in the group additionally treated with progestagen/PGF2α/eCG (94.4% vs. 69.4%). Overall, the percentage of lambing ewes was similar in both groups (97.2% and 94.4%), and lambing rates (1.4–1.9) and litter sizes (1.9–2.1) were high and not influenced ($P > 0.05$) by the treatment. In conclusion, this study reports that exposition of Lacaune ewes to artificial long days followed by natural day length and male introduction is highly effective to induce fertile estrous activity during the nonbreeding season and offers a reliable and practical alternative to hormonal manipulation for out-of-season breeding in sheep.

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1. Introduction

Reproduction in sheep follows a seasonal pattern, which leads to variations in the availability of products throughout the year. To fulfill consumers' demands for milk and meat all year round, out-of-season breeding is required. Hormonal estrus induction with progestagens results in a highly synchronous estrus but a variable fertility [1] and has the disadvantage of additional costs and labor. Additionally, societal constrains, animal welfare, and consumer's demand of products that are "clean" encourage producers to minimize or completely avoid pharmacologic manipulation [2].

In sheep and goats, the introduction of males in a flock of anestrous females provokes an increase in LH pulsatility followed by synchronized ovulations [3]. This phenomenon, called "male effect", works well in Merino sheep for induction of fertile estrus in the nonbreeding season; however, in other breeds, it is less effective [2]. Photoperiod is the crucial factor driving reproductive seasonality [4]. Small ruminants are short-day breeders, and decreasing day length promotes the seasonal onset of cycling activity. Light programs consist of an alternation of long days, to restore the receptivity for the following stimulatory short days [5,6]. In ewes, long days are provided by artificial illumination, whereas short days are simulated through melatonin implants [7,8]. As an alternative to the melatonin application, the exposition to naturally short day length has been shown to also be effective in the goat [8–10]. In dairy sheep, however, there are no published reports on the efficacy of light programs without melatonin application to induce cycling activity during the nonbreeding season. Therefore, the aim of this study was to evaluate the effect of photoperiodic manipulation in conjunction with the male effect on reproductive performance in Lacaune dairy sheep during the anestrous season. Furthermore, efficacy was compared with ewes treated additionally with progestagen sponges in combination with PGF2α and eCG.

2. Materials and methods

2.1. Animals

The experiment was carried out with 72 Lacaune ewes and four rams on a commercial farm in the eastern part of Switzerland (47° 22′N, 9° 4′E). All ewes were pluriparous, aged between 2 and 10 years, weighing around 65 to 75 kg, and were 176 to 250 days in milk at joining. The animals were kept in a barn with daily access to a paddock and fed hay and concentrate. Mean milk production was 400 kg over a lactation period of 270 to 300 days.

2.2. Light program, estrus induction, and breeding

The manipulation of photoperiod was carried out in winter during 77 days from December 15 until March 2. During this period, day length was extended to 16 hours by additional artificial lighting from 6 to 9 AM and from 4 to 10 PM. The light exposition at sheep-eye level reached an intensity of at least 100 lux everywhere in the barn. After the light program, the ewes were exposed to natural day length (9.5 hours day light on March 2). The rams were kept in a separated barn and not exposed to the light program. The flock was randomly assigned to two groups of 36 ewes and two rams each. The ewes of one group were separated in a pen and additionally treated with intravaginal sponges containing 20-mg fluorogestone acetate (Chronogest CR; MSD Animal Health) for 6 days. At insert removal, 0.125 mg of the prostaglandin analog cloprostenol (Estrumate; MSD Animal Health) and 500 IU of eCG (Folligon; MSD Animal Health) were administered intramuscularly. Progestagen treatment was carried out 33 to 43 days after the end of the light program. To prevent overstraining of the rams, the sponges were inserted on five consecutive days (April 4–8), and no more than four ewes were treated per ram and day. Progestagen-treated sheep were placed together with rams fitted with marking harnesses 24 hours after insert removal (April 11–15). Ewes exposed to the light program only were joined 40 days (April 11) after the end of photostimulation. Estrus detection was performed twice daily at milking time for 45 days, and ewes marked by the rams were recorded.

2.3. Fertility

In autumn, lambing dates and the number of lambs born per ewe were recorded, and gestation length was determined. The parameter "ewes lambing" was defined as the number of females lambing expressed as a percentage of treated ewes. Lambing rate was calculated by dividing the total number of lambs born by the number of treated ewes, and litter size was determined by dividing the total number of lambs born by the number of ewes that lambed.

2.4. Blood sampling and progesterone (P4) determination

At the end of the light program, three blood samples were collected 6 days apart (February 18 and 24 and March 2) from every ewe by puncture of the jugular vein using vacutainers (9-mL Z Serum Clot Activator Vacuette; Greiner Bio-One GmbH, Kremsmünster, Austria). The samples were allowed to clot during 2 hours at room temperature. After centrifugation ($\times$4000*g*, 10 minutes), serum was frozen and stored at -18 °C until analysis. Serum P4 concentrations were determined using a commercially available Coat-A-Count RIA Kit (Progesterone Coat-a-Count, TKPG1; Siemens Medical Diagnostics, CA, USA) according to the instructions provided by the manufacturer. The analytical specificity was 100% for P4 with the following cross-reactivities: 9.0% for 5α-Pregnan-3,20-dione, 3.4% for hydroxy P4, 3.2% for 5β-Pregnan-3,20-dione, 2.2% for 11-deoxycorticosterone, and 0.9% for corticosterone. The analytical sensitivity was 0.02 ng/mL, and the intra-assay coefficient of variation was 4.0%. Progesterone concentrations of $\geq$1 ng/mL were considered as indicative of ovulatory activity [11]. Ewes with P4 concentrations less than 1 ng/mL in all the three blood samples were classified as noncyclic.

2.5. Statistical analysis

The data were analyzed using R: A language and environment for statistical computing (R Foundation for

Statistical Computing, Vienna, Austria; www.r-project.org) version 3.0.1. Normally (percentages of ewes lambing) and non-normally distributed data (lambing rate, litter size, and P4 concentrations) were compared using the Fisher exact test and the Mann–Whitney *U* test, respectively. P values were considered as significant when less than 0.05. Ewes in estrus are presented as percentages, lambing rate, litter size, and P4 concentrations as mean ± standard deviation.

3. Results

3.1. Estrus response and distribution

The estrus response was 97.2% in both the groups, and only one ewe in each group was not marked by the rams during the 45-day observation period (Table 1). The first estrus in ewes exposed to the light program only was noted within the first 4 days after joining (6 ewes), from Days 8 to 17 (17 ewes) and from Days 19 to 25 (12 ewes) after ram introduction. A second estrus was observed in one ewe 32 days after joining (Fig. 1A).

In progestagen-treated ewes, 75.0% (27 ewes) and 91.7% (33 ewes) of the animals displayed the first estrus on Day 2 and within 4 days after insert removal, respectively. Two other ewes were marked by the rams on Days 18 and 26 after sponge withdrawal. A second estrus was recorded in 10 ewes on Days 8 (n = 1), 18 (n = 3), 19 (n = 4), and 20 (n = 2) after progestagen withdrawal (Fig. 1B).

3.2. Fertility

Reproductive data of ewes treated with a light program with or without additional progestagen/PGF2α/eCG treatment are summarized in Table 1. Lambings were recorded between 145 and 179 days (lambing period 35 days) after male introduction in ewes exposed to the light program only and between 146 and 172 days (lambing period 27 days) after insert removal in ewes additionally treated with progestagen/PGF2α/eCG. Gestation length varied between 143 and 154 days (mean = 147.5 ± 1.9 days). Treatment had an effect ($P < 0.05$) on the percentage of ewes lambing to the first service (94.4% vs. 69.4%) but not overall (97.2% vs. 94.4%). No group differences were present in lambing rate and litter size.

Table 1
Reproductive data of Lacaune ewes exposed to a light program without (n = 36) or with (n = 36) an additional 6-day progestagen treatment in combination with PGF2α and eCG at insert removal during the nonbreeding season.

Reproductive parameter	Light program only	Light program and progestagen/PGF2α/eCG
Estrus response	35/36 (97.2%)	35/36 (97.2%)
Ewes lambing		
First estrus	34/36 (94.4%)[b]	25/36 (69.4%)[a]
Overall	35/36 (97.2%)	34/36 (94.4%)
Lambing rate		
First estrus	1.9 ± 0.7	1.4 ± 1.2
Overall	1.9 ± 0.6	1.8 ± 0.9
Litter size		
First estrus	2.0 ± 0.5	2.1 ± 0.9
Overall	2.0 ± 0.5	1.9 ± 0.8

[a,b]Different letters within the same row and ewe subgroup indicate significant ($P < 0.05$) differences.

3.3. Cyclicity and P4 concentrations

The mean serum P4 concentrations of the three samples taken 6 days apart at the end of the light program are represented in Figure 2. No differences ($P > 0.05$) were evident between the groups. Serum P4 concentrations of 1 ng/mL or greater, an indicator of ovulatory activity, were measured in 27.8% (20 of 72) of the ewes at the end of the light program. On the basis of P4 concentrations less than 1 ng/mL in all the three blood samples collected 6 days apart, 72.2% (52 of 72) of ewes were classified as noncyclic. No difference ($P > 0.05$) in the proportion of noncyclic ewes between treatment groups was apparent (25 of 36, 69.4% of ewes with light program only; 27 of 36, 75.0% of ewes with light program and progestagen/PGF2α/eCG treatment). Within treatment groups, no differences ($P > 0.05$) between cyclic and noncyclic ewes at the end of the light program were evident in the evaluated fertility parameters.

4. Discussion

The results of this study report that exposure of Lacaune dairy ewes during winter to a light program with subsequent male introduction leads to a reliable estrus induction and high fertility outside the breeding season.

The estrus response was equally high (97.2%) in both treatment groups but less synchronous than expected in ewes treated with the light program only. After introduction of the ram in a flock of anovulatory ewes, a two-peak pattern of estrous activity between 18 to 20 days and 24 to 26 days after joining has been reported in the literature [3,12]. In this study, however, estrous activity was noted in six ewes already within 4 days and in the remaining 29 ewes between 7 and 24 days after joining. The early occurrence of estrus could be explained by ewes with cycling activity before male introduction. In cyclic ewes, exposition to a ram can advance the LH surge [13], shorten the follicular phase [14], and even cause early luteolysis, but no ovulation can be induced [15]. However, the high estrous activity around Day 10 after joining indicates that ovulation did occur in most ewes immediately after exposure to the rams and that the following luteal phase was shortened. As it is known that the occurrence of short luteal cycles is negatively correlated to the percentage of females cycling before joining [3] and because persistently low P4 levels were measured at the end of the light program in more than 70% of the ewes, it can be assumed that most of the sheep were noncyclic before. In a study [16] performed 1 year before with the same flock in spring, nearly half of the pluriparous ewes showed elevated serum P4 concentrations. These results indicate that reproductive activity in Lacaune sheep is not strictly seasonal as reported earlier [11]. However, previous breeding attempts in this flock in spring were not successful, estrus response after male introduction was only moderate, and no lambings were recorded. Moreover, it seems that in Lacaune, unlike in other seasonal sheep

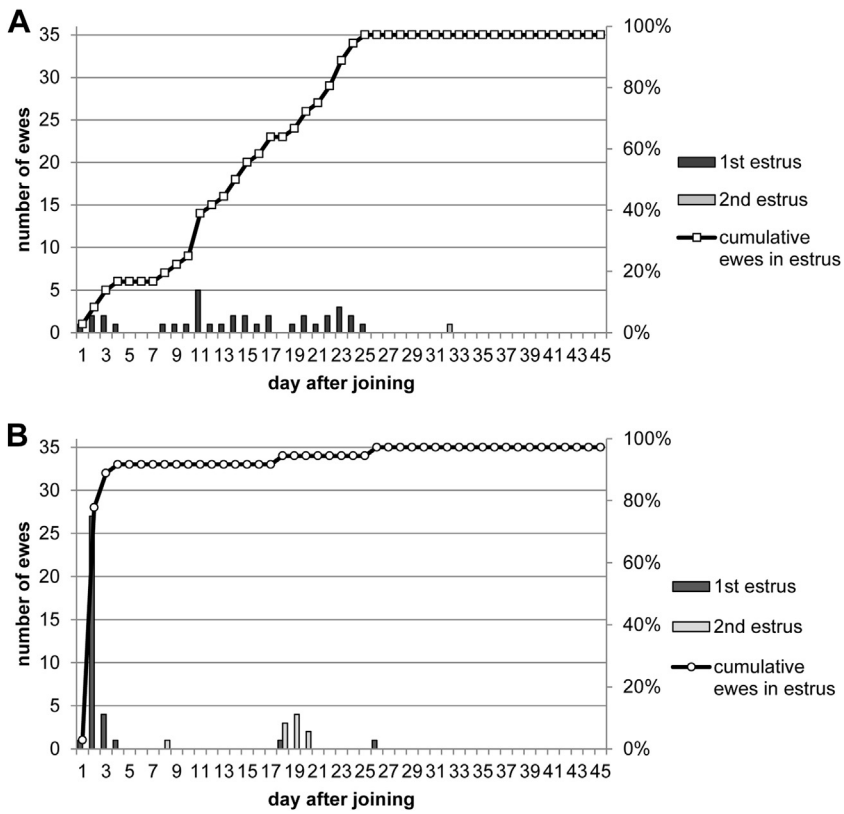

Fig. 1. Estrus distribution of Lacaune ewes exposed to a light program without (A, n = 36) or with additional 6-day progestagen treatment in combination with PGF2α and eCG at insert removal (B, n = 36) during the nonbreeding season.

breeds [3], but similar to goats [17,18], estrous behavior can be expressed without a previous luteal phase of normal duration.

The estrus rate achieved in the progestagen-treated ewes was similar (91.7% within 96 hours) to the results reported by Ungerfeld and Rubianes [19] but higher (82%–96%) than those obtained by Knights et al. [20], both using short-term progestagen treatments during the anestrous season. Estrus activity was highly synchronous, and most ewes were in estrus 48 to 72 hours after sponge removal, which is slightly later than the reported 38.8 ± 1.6 hours after a 6-day priming with fluorogestone acetate and eCG [19].

The overall percentage of ewes lambing was, independent of the treatment, very high (97.2% and 94.4%) in both groups. Regarding the first estrus, however, the results were significantly lower in ewes additionally subjected to a progestagen/PGF2α/eCG treatment (69.4% vs.

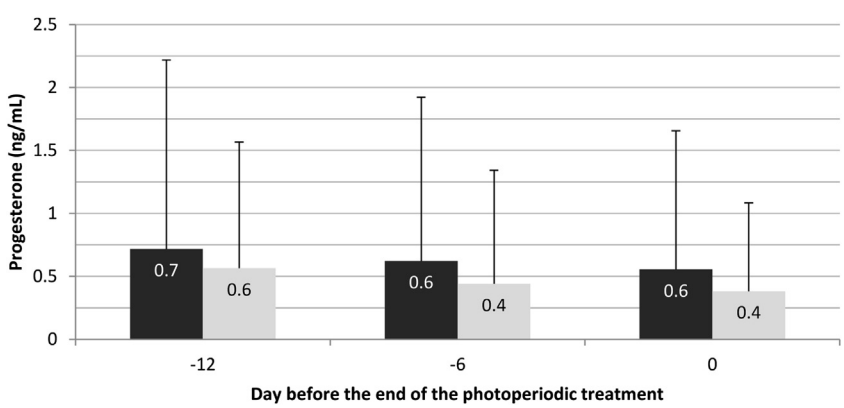

Fig. 2. Serum progesterone concentrations (mean ± standard deviation) of the treatment groups (ewes with light program only, n = 36; ewes with light program and additional 6-day progestagen treatment in combination with PGF2α and eCG, n = 36) at the end of the photoperiodic treatment.

94.4%). A reduced fertility after progestagen treatment compared with natural estrus has already been reported [21,22]. Possible explanations for the reduction of fertility in progestagen-treated animals are alterations in final follicle growth [23] and ovulation [22–24] as well as impairment of sperm transport and survival in the female reproductive tract, reducing the number of fertilized ova [25,26]. When comparing the percentage of ewes lambing to the first estrus after progestagen treatment in this investigation with that of a study [16] performed the previous year with the same flock, the results are almost equal (69.4% vs. 75%). In earlier reports using progestagen medications, similar (71%) [27] or clearly lower (46%) [20] values were found.

Regarding lambing rate and litter size, no difference could be found between treatments. This observation is surprising because the ewes treated with progestagens also received 500 IU eCG at sponge removal. Treatment with eCG is known to enhance the recruitment of small follicles [28], to improve the synchrony of estrus [29,30], to increase ovulation rates during the breeding season [28,29,31], and to enable the induction of estrous activity during the anovulatory season [32]. In this study, lambing rates (1.8–1.9) and litter sizes (1.9–2.0) were higher than those reported in previous investigations (0.7–1.1 and 1.1–1.7, respectively) [20,27,33]. The main reason for the high prolificacy achieved even without eCG treatment was probably that this experiment was performed only with multiparous and well-managed and conditioned ewes.

The light program used in conjunction with male introduction used in this study has been proven to be highly effective to induce fertile estrus in anestrous Lacaune sheep. The program was based on research in goats [8,10,34] and consisted of a period of 77 lengthened days during winter followed by natural photoperiod and ram introduction 40 days after the end of photostimulation. In the end of winter, seasonal sheep become refractory to short day lengths; therefore, exposition to a prolonged period of artificial long days is necessary to restore receptivity before short days, or melatonin implants can be applied to stimulate reproductive activity. For photostimulation, light intensities of 100 to 200 lux at eye level are sufficient [34,35], and for an optimal use of the male effect, ewes have to be joined between 35 and 70 days after the beginning of the short-day stimulation [10,34]. Induction of fertile ovarian activity in anovulatory ewes using the male effect without previous photostimulation is only effective in breeds with moderate seasonality, such as the Merino sheep [36]. In highly seasonal breeds, however, the onset of the breeding season can be advanced by the male effect for a few weeks, but no induction of estrous activity in the anestrous season is possible [37].

In conclusion, this study reports that exposing Lacaune ewes to artificial long days during winter followed by natural day length and male introduction was highly effective to induce fertile estrous activity during the nonbreeding season. This method offers a reliable and practical alternative to hormonal manipulation for out-of-season breeding programs in sheep.

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